“Exploration of Acarbose-Producing Bacteria for Glycosidase Inhibition in Therapeutic"

Synopsis submitted to

Swami Ramanand Teerth Marathwada University, Nanded

For

Registration to Doctor of Philosophy (Microbiology)

Under the faculty of Science

By

Ms. Gawali Pornima Panditrao

Under the Guidance of

Prof. Dr.Smt. D.C. Kamthane.

Department of Microbiology

Shri Guru Buddhiswami Mahavidyalaya,

Purna (Jn.),Dist.Parbhani

Dec 2024

INDEX

Sr.No.	Content
1.	Introduction
2.	Review of Literature
3.	Aim & Objectives
4.	Methodology
5.	Importance of Study/Society application
6.	Proposed Work Plan/Formulation & Structure of Study
7.	References

Introduction

Acarbose is an important pharmaceutical agent used in the management of type 2 diabetes. It is a potent α-glucosidase inhibitor, which works by delaying the breakdown of complex carbohydrates into glucose in the small intestine. This results in reduced postprandial blood glucose spikes, making it an effective treatment for controlling hyperglycemia in diabetic patients. Acarbose, however, is not synthetically manufactured but is derived from microorganisms, particularly acarbose-producing bacteria, which play a crucial role in its large-scale production. These bacteria, mainly *Actinoplanes utahensis* and related species, are renowned for their ability to synthesize acarbose through complex biosynthetic pathways involving various enzymes.

The production of acarbose by *Actinoplanes* species has been of significant interest in biotechnology due to its potential for large-scale fermentation processes. These microorganisms naturally produce acarbose as a secondary metabolite under specific growth conditions, which are optimized for industrial production. Understanding the genetic and biochemical pathways involved in acarbose biosynthesis has allowed researchers to enhance yields, making acarbose more accessible for medical use.

Acarbose's therapeutic effect is based on its ability to inhibit α-glucosidase, an enzyme responsible for the hydrolysis of oligosaccharides and disaccharides into absorbable monosaccharides, particularly glucose. By binding to the active site of the enzyme, acarbose slows down the digestion of carbohydrates, thus reducing the absorption of glucose and helping to prevent the sharp rise in blood sugar levels following meals. This mechanism of action aligns with the broader class of glycosidase inhibitors, which are a key target for drug development in the treatment of diabetes and other metabolic disorders.

Recent research has expanded beyond acarbose to explore other glycosidase inhibitors, which may offer benefits such as improved potency, selectivity, and reduced side effects. Despite the widespread use of acarbose and its derivatives like miglitol and voglibose, challenges remain in optimizing the production processes and enhancing the efficiency of glycosidase inhibition. Moreover, the ongoing exploration of microbial strains capable of producing acarbose at higher yields continues to be a focus in biotechnology, addressing the growing demand for this essential drug.

Review of Literature

1.**Christoph Rosak and Gabriele Mertes (2012)** his critically evaluated the role acarbose in the treatment of diabetes, emphasizing patient considerations. Their work highlights the increasing global prevalence of diabetes, particularly type 2 diabetes, and the importance of early diagnosis to reduce complications. They discuss acarbose, an alpha-glucosidase inhibitor used for over 20 years in managing type 2 diabetes and prediabetes, especially in its early stages, as a pharmacological option when lifestyle changes alone are insufficient for glycemic control.

2.**Ekundayo F. O et.al (2023).** conducted a study screening seven *Actinoplanes* species isolated from termitary soils for their ability to produce acarbose. Among the species, *Actinoplanes utahensis* showed the highest acarbose activity. The researchers also observed a significant reduction in blood glucose levels in experimental rats, from over 250 mg/dL before treatment to 115 mg/dL after treatment. Additionally, hematology results showed notable changes in red blood cell, white blood cell, lymphocyte, and platelet counts, with improvements towards normalcy after treatment.

3.**Gernot Vobis** contributed to the study of the genus *Actinoplanes* and its related genera, detailing their biology in the handbook "A Handbook on the Biology of Bacteria," specifically in volume 3, pages 623-653, published in **2006**.

4.Masayuki Hayakawa (2008) developed various methods for isolating desirable rare actinomycete genera from natural habitats, in collaboration with Nonomura and others. His work includes developing pretreatment and enrichment techniques, often supplemented with selective antimicrobial agents, for isolating genera such as *Actinoplanes*, *Actinokineospora*, *Acrocarpospora*, *Actinosynnema*, and many more. The paper also reviews the occurrence and distribution of these rare actinomycetes in Japanese soils.

5.Patrick Schwientek conducted research on the genomics and transcriptomics of the industrial acarbose producer *Actinoplanes* sp. SE50/110. His Ph.D. thesis, submitted to the Faculty of Technology at Bielefeld University, Germany, in January **2012,** contributed to understanding the genetic and transcriptional mechanisms underlying acarbose production in this strain.

6.Sai-jin Wei et al (2010) conducted research to optimize the fermentation medium for *Actinoplanes* sp. A56 to enhance acarbose production. Using fractional factorial design, steepest ascent methodology, and response surface analysis, they identified and refined key factors, including maltose and corn steep liquor, resulting in an increase in acarbose yield from 837 to 1043 mg/L.

7.Ya-Jun Wang et al. (2011) investigated acarbose production using the overproducing strain *Actinoplanes utahensis* ZJB-08196. They optimized fermentation conditions, achieving a yield of 4,210 mg/L, and identified maltose, glycerol, and monosodium glutamate as supportive substrates, with soybean meal showing superior bioavailability over corn steep liquor. Their findings also revealed that acarbose production followed a mixed-growth-associated pattern, independent of mycelial growth.

8.Michael Goodfellow (2010) has contributed significantly to the exploration of microbial diversity for biotechnology, focusing on the isolation and characterization of previously

uncultivated prokaryotic taxa, particularly within the order *Actinomycetales*. His work highlights the importance of *Streptomyces* strains and other actinobacteria as prolific sources of novel bioactive compounds, including antibiotics, and emphasizes the need for innovative strategies to access the untapped genetic diversity of actinobacteria in natural habitats.

9.**Kun-tai Li et al. (2012)** conducted research to optimize industrial acarbose production by *Actinoplanes* sp. A56. Their study identified ideal fermentation conditions, including high sugar concentration, specific pH levels, and dissolved oxygen levels, resulting in an improved acarbose yield of approximately 5000 mg/L after 168 hours of fermentation

10.Van Bon Nguyen et al. (2017) conducted research on Paenibacillus sp. TKU042, a bacterium isolated from Taiwanese soil, which produced α-glucosidase inhibitors with stronger inhibitory activities than the commercial anti-diabetic drug acarbose. Their findings demonstrated the potential of fermented nutrient broth (FNB) from Paenibacillus sp. TKU042 as a stable, effective, and multifunctional treatment for type 2 diabetes, with added benefits in animal models, including reduced plasma glucose levels and improved tolerability.

11.Nguyen Dương et al. (2016) worked on optimizing the fermentation medium for acarbose production using the *Actinoplanes* sp. KCTC 9161 – L14 mutant strain. They employed fractional factorial design to investigate the effects of glucose, maltose, and corn powder on acarbose production, identifying glucose and maltose as key factors. Additionally, they developed a regression model and successfully demonstrated that chromatography using an activated charcoal column could purify acarbose from the fermentation broth, achieving a yield of 191.5 g/L of acarbose in the purified solution.

12.**Rahul B.et al.** (**2020**) identified an alpha-glucosidase inhibitory compound from the marine bacterium *Arthrobacter enclensis*. The purified compound, resembling acarbose, was analyzed using HPLC, FTIR, and tandem mass spectrometry, and matched biosynthetic gene clusters of acarviostatin and acarbose. The compound showed inhibitory activity with an IC50 value of 500 µg/ml, highlighting its potential as a therapeutic agent for diabetes and obesity.

13.Werner Creutzfeldt (1999) conducted studies demonstrating the short-term efficacy of acarbose in reducing postprandial blood glucose and glycated hemoglobin (HbA1c) levels, with promising implications for mitigating long-term diabetes complications. His research, including 18 animal studies, highlighted acarbose's potential in delaying or preventing renal, retinal, lens, neurological changes, and ischemic myocardial lesions, offering benefits for patients with Type 2 diabetes and, in combination with insulin, Type 1 diabetes.

14.**Patrick Schwientek et al. (2012)** collaborated to present the complete genome sequence of *Actinoplanes* sp. SE50/110, the first publicly available genome from the genus *Actinoplanes*. Their research revealed significant features, including a high G+C content, biosynthetic gene clusters for acarbose and other bioactive compounds, and a fun actinomycete integrative and conjugative element (AICE), which holds potential for genetic optimization of acarbose production and the discovery of novel antibiotics.

Aims & Objectives of Research

Aims of Research:

To enhance understanding and innovation in the field of acarbose production, glycosidase inhibition, and the therapeutic use of these inhibitors in managing diabetes.

Objectives:

Objectives are as follows:

1. Identify and characterize bacterial strains producing acarbose.
2. Optimize fermentation conditions for maximal acarbose yield.
3. Assay the inhibitory activity of bacterial acarbose against glycosidase enzymes.
4. Explore potential therapeutic applications of acarbose-producing bacteria.

Methodology:

1. Selection and Isolation of Bacteria

1. Collect soil samples from diverse habitats (e.g., rhizospheres, forest soils).
2. Isolate actinobacteria, particularly *Actinoplanes* species, using selective media like starch-casein agar.
3. Perform morphological and biochemical characterization to identify potential acarbose-producing strains.

2. Screening for Acarbose Production

1. Inoculate the bacterial isolates into fermentation media optimized for acarbose production (e.g., media containing maltose, starch, or glucose as carbon sources).
2. Harvest the culture supernatant at regular intervals.
3. Use high-performance liquid chromatography (HPLC) or thin-layer chromatography (TLC) to detect acarbose in the supernatant.

3. Optimization of Fermentation Conditions

1. Optimize parameters like pH, temperature, agitation, and aeration in the fermentation process.
2. Investigate the effect of different carbon and nitrogen sources.
3. Scale up production using bioreactors once optimal conditions are determined.

4. Extraction and Purification of Acarbose

1. Extract acarbose from the fermentation broth using adsorption chromatography.
2. Purify the compound using techniques like ion-exchange chromatography or reverse-phase HPLC.
3. Confirm purity using spectroscopic methods (e.g., NMR).
4.

5. Glycosidase Inhibition Assays

1. Prepare glycosidase enzyme solutions (e.g., α-glucosidase).
2. Incubate the enzyme solutions with purified acarbose at varying concentrations.
3. Measure enzymatic activity using chromogenic substrates.
4. Calculate the inhibition constant (IC50) to evaluate potency.

6. In Vivo Studies

1. Use animal models to study the effect of acarbose on postprandial blood glucose levels.
2. Side effects.

7. Genomic and Molecular studies.

1. Sequence the genome of the acarbose-producing bacteria.

Importance of study/Society application

National ,International Significance of Study are as follows:

1. Advancing Medical Science

- **Diabetes Management**: Acarbose is widely used to manage type 2 diabetes by slowing carbohydrate digestion and reducing postprandial glucose spikes. Research on bacterial production can make this drug more accessible and affordable.
- **Novel Therapeutics**: Identifying new strains or optimizing bacterial production pathways can lead to enhanced formulations with improved efficacy or reduced side effects compared to synthetic glycosidase inhibitors.

2. Cost-Effective and Sustainable Drug Production

- **Biotechnological Advantage**: Utilizing bacteria for acarbose production can lower production costs compared to chemical synthesis, making diabetes medications more affordable, especially in low- and middle-income countries.
- **Environmental Sustainability**: Bacterial fermentation offers an eco-friendly alternative to synthetic drug production, reducing reliance on chemical reagents and minimizing waste.

3. Potential as Probiotics

- **Gut Health**: Acarbose-producing bacteria could be developed into probiotics that naturally regulate glycemic levels while simultaneously promoting gut microbiome health.
- **Multifunctional Benefits**: These bacteria may offer dual benefits by acting as glycosidase inhibitors and contributing to the maintenance of a balanced gut microbiota.

4. Contribution to Metabolic Disease Research

- **Understanding Enzyme Inhibition**: Studying the interaction between acarbose and glycosidase enzymes provides valuable insights into carbohydrate metabolism, paving the way for new treatments for other metabolic disorders.

- **Exploring Microbial Biosynthesis**: Research into acarbose biosynthesis expands our understanding of secondary metabolites, which could lead to the discovery of novel inhibitors for other enzymes.

5. Industrial and Pharmaceutical Applications

- **Scalable Production**: Optimizing bacterial production of acarbose can support large-scale industrial applications, ensuring a steady and sustainable supply for global healthcare needs.
- **Bioengineering Opportunities**: Genetically modifying bacteria to enhance acarbose production could result in the development of tailored inhibitors for specific glycosidase enzymes.

6. Global Health Impact

- **Addressing the Diabetes Epidemic**: Diabetes affects millions worldwide, and affordable treatment options like acarbose are crucial for managing this chronic condition.
- **Accessibility**: By reducing the cost of production, bacterial synthesis of acarbose could make treatment available to underserved populations.

7. Promoting Interdisciplinary Innovation

- **Integration of Biotechnology and Medicine**: This research bridges the gap between microbial biotechnology and therapeutic medicine, fostering innovation in both fields.
- **Application of Genomics and Proteomics**: Insights from genomic and proteomic studies of acarbose-producing bacteria may lead to breakthroughs in other therapeutic areas.

8. Education and Knowledge Dissemination

- **Scientific Literacy**: This study helps in educating researchers, students, and the public about the role of bacteria in drug production and metabolic health.
- **Inspiration for Future Research**: It serves as a model for exploring microbial biosynthesis of other valuable therapeutic compounds.

Proposed Work Plan/Formulation & Structure of Study

The study will follow a stepwise approach:

A. Literature Review

- Study the existing research on acarbose producing bacteria, glycosidase inhibitors, and bacterial secondary metabolites.
- Identify gaps in knowledge to refine research questions.

B. Sample Collection and Strain Isolation

- **Tasks**:
  - Collect environmental samples from soil, compost, or other natural habitats rich in microbial diversity.
  - Isolate potential acarbose-producing bacteria using selective media and screening assays.
- **Expected Outcome**: A collection of bacterial isolates, with initial identification using morphology and basic biochemical tests.

C. Molecular and Genetic Identification

- **Tasks**:
  - Perform 16S rRNA sequencing or whole-genome sequencing to identify bacterial strains.
  - Compare with known acarbose producers such as *Actinoplanes sp.*
- **Expected Outcome**: Identification of bacterial species with potential acarbose production capability.

D. Fermentation and Optimization

- **Tasks**:
  1. Develop fermentation conditions for bacterial growth and secondary metabolite production.
  2. Optimize factors such as carbon/nitrogen sources, pH, temperature, and aeration.
- **Expected Outcome**: High-yield production of acarbose in controlled conditions.

E. Extraction and Purification of Acarbose

- **Tasks**:
  - Use solvents and chromatographic techniques (HPLC, TLC) to extract and purify acarbose.
  - Confirm the structure using spectroscopy (e.g., NMR,).
- **Expected Outcome**: Pure acarbose ready for inhibitory activity assays.

F. Glycosidase Inhibition Assays

- **Tasks**:
  - Test purified acarbose against glycosidase enzymes such as α-glucosidase or α-amylase.
  - Measure enzyme inhibition using spectrophotometric assays.
  - Calculate IC50 values to evaluate inhibitory potency.
- **Expected Outcome**: Quantification of glycosidase inhibition efficiency.

G. Application Studies

- **Tasks**:
  - Test the therapeutic potential of acarbose in vitro (enzyme assays) and in vivo (animal models)
- **Expected Outcome**: Data supporting the practical use of acarbose in medicine and industry.

H. Data Analysis and Reporting

- **Tasks**:
  - Analyze all experimental data statistically to ensure validity.
  - Prepare comprehensive reports and manuscripts for publication.

Expected Outcome: Peer-reviewed publications and contributions to scientific knowledge

Year-Wise Plan of work and targets to be achieved

Sr.No.	Year	Activity/Targets	Tentative Duration
1	First Year	a..Literature Review b.Methodology c.Sample Collection and Isolation d.Identification using Morphology & Biochemical test.	12 Months
2	Second Year	a.Molecular and Genetic Identification b.Fermentation and Optimization c.Extraction & Purification d.Inhibition Assays. e.Data Collection and Analysis.	12 Months
3	Third Year	a.Application Studies b.Data Analysis and Reporting c.Prepare comprehensive reports and manuscripts for publication d.Thesis Preparation.	12 Months

References

1.Actinoplanetes, S. (2006). The genus Actinoplanes and related genera. A Handbook on the Biology of Bacteria, 3, 623-653.

2.Choi, B. T., & Shin, C. S. (2003). Reduced Formation of Byproduct Component C in Acarbose Fermentation by Actinoplane ssp. CKD485–16. *Biotechnology progress*, *19*(6), 1677-1682.

3.Creutzfeldt, W. (1999). Effects of the α-glucosidase inhibitor acarbose on the development of long-term complications in diabetic animals: pathophysiological and therapeutic implications. Diabetes/metabolism research and reviews, 15(4), 289-296.

4.Ekundayo, F. O., Olabanji, B. O., Adedayo, B. C., Akindele, A. M., & Efe, I. L. WITHDRAWN: Production of Acarbose From Actinoplanes Species Isolated From Termite Mound for Treatment of Type Two Diabetes.

5.Hanefeld, M. (1998). The role of acarbose in the treatment of non–insulin-dependent diabetes mellitus. Journal of Diabetes and its Complications, 12(4), 228-237.

6.Hayakawa, M. (2008). Studies on the isolation and distribution of rare actinomycetes in soil. Actinomycetologica, 22(1), 12-19.

7.Hayakawa, M., Tamura, T., & Nonomura, H. (1991). Selective isolation of Actinoplanes and Dactylosporangium from soil by using γ-collidine as the chemoattractant. *Journal of fermentation and bioengineering*, *72*(6), 426-432.

8.Jiang, Y., Li, Q., Chen, X., & Jiang, C. (2016). Isolation and cultivation methods of Actinobacteria. *Actinobacteria-basics and biotechnological applications*, *2*, 39-57.

9.Li, K. T., Zhou, J., Wei, S. J., & Cheng, X. (2012). An optimized industrial fermentation processes for acarbose production by Actinoplanes sp. A56. Bioresource technology, 118, 580-583

10.Manivasagan, P., Venkatesan, J., Sivakumar, K., & Kim, S. K. (2015). Actinobacterial enzyme inhibitors–A review. Critical reviews in microbiology, 41(2), 261-272.

11.Mawlankar, R. B., Dharne, M. S., & Dastager, S. G. (2020). Isolation of potent alpha-glucosidase inhibitor from a novel marine bacterium Arthrobacter enclensis. SN Applied sciences, 2, 1-11.

12.Nguyen, V. B., Nguyen, A. D., Kuo, Y. H., & Wang, S. L. (2017). Biosynthesis of α-glucosidase inhibitors by a newly isolated bacterium, Paenibacillus sp. TKU042 and its effect on reducing plasma glucose in a mouse model. International journal of molecular sciences, 18(4), 700.

13.Schwientek, P. (2012). Genomics and transcriptomics of the industrial acarbose producer Actinoplanes sp. SE50/110.

14.Schwientek, P., Szczepanowski, R., Rückert, C., Kalinowski, J., Klein, A., Selber, K., ... & Pühler, A. (2012). The complete genome sequence of the acarbose producer Actinoplanes sp. SE50/110. BMC genomics, 13, 1-18.

15.T. H., & Do, T. T. (2016). Optimization of culture medium for the cultivation of Actinoplanes sp. mutant strains and purification of acarbose. Journal of Vietnamese Environment, 8(1), 14-20.

16.Wang, Y. J., Liu, L. L., Feng, Z. H., Liu, Z. Q., & Zheng, Y. G. (2011). Optimization of media composition and culture conditions for acarbose production by Actinoplanes utahensis ZJB-08196. World Journal of Microbiology and Biotechnology, 27, 2759-2766.

17.Wei, S. J., Cheng, X., Huang, L., & Li, K. T. (2010). Medium optimization for acarbose production by Actinoplanes sp. A56 using the response surface methodology. African Journal of Biotechnology, 9(13), 1949-1954.

18.Wendler, S., Ortseifen, V., Persicke, M., Klein, A., Neshat, A., Niehaus, K., ... & Pühler, A. (2014). Carbon source dependent biosynthesis of acarbose metabolites in Actinoplanes sp. SE50/110. *Journal of Biotechnology*, *191*,113-120.

19.Xue, Y. P., Qin, J. W., Wang, Y. J., Wang, Y. S., & Zheng, Y. G. (2013). Enhanced Production of Acarbose and Concurrently Reduced Formation of Impurity C by Addition of Validamine in Fermentation of Actinoplanes utahensis ZJB-08196. *BioMed research international*, *2013*(1), 705418

20.Zhao, Q., Xie, H., Peng, Y., Wang, X., & Bai, L. (2017). Improving acarbose production and eliminating the by-product component C with an efficient genetic manipulation system of Actinoplanes sp. SE50/110. Synthetic and Systems Biotechnology, 2(4), 302-309

Signature

Ms.Gawali Pornima Panditrao

Research Student

Signature

Prof. Dr.Smt. D.C. Kamthane

Research Guide